J. Biol. Chem. **218**, 293 (1956)] were used: I, isopropyl alcohol-ammonia (5 percent), 8/2; II, benzene-propionic acid-H_2O, 2/2/1; III, *n*-butanol-acetic acid-H_2O, 8/2/2. Phenolic compounds were visualized by spraying the chromatograms with diazotized *p*-nitroaniline [H. G. Bray, W. V. Thorpe, K. White, *Biochem. J.* (*London*) **46**, 271 (1950)]

8. S-Adenosylmethionine and 3-methoxy-4-hydroxymandelic acid were kindly supplied by G. L. Cantoni and M. S. Fish, respectively, National Institutes of Health.
9. Enzymatically formed methoxy epinephrine and the metabolite isolated from the urine after the administration of l-epinephrine bitartrate had the same R_f, fluorescent spectrum, and partition coefficients as a synthetic sample of 3 methoxy-4-hydroxyphenyl-2-methylaminoethanol (synthesized by S. Senoh and B. Witkop, National Institutes of Health).
10. The solvent systems sharply separated methoxy epinephrine and methoxy norepinephrine.
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26 July 1957

Isolation of Cytopathogenic Viral Agent from Feces of Cattle

In the early part of September 1956, 19 beef calves, 3 to 4 months of age, were purchased and brought into an experimental barn unit. Approximately 10 days later the calves developed temperatures varying from 104° to 106°F, and several also had a slight cough and some nasal exudate. The calves were treated with broad-spectrum antibiotics and recovered uneventfully.

Two pregnant cows were brought into the same barn unit on 7 October. These cows developed fever and nasal exudate within a week. One cow recovered in a few days but aborted approximately 1 month later. The other cow developed pulmonary complications and was treated with broad-spectrum antibiotics over a period of 21 days. When it was examined 2 to 3 months later, this cow was not pregnant. The aborted 7-month-old fetus and both cows were found to be negative for evidence of infection caused by *Vibrio fetus, Leptospira pomona,* and *Brucella abortus* (*1*).

On 5 January 1957, nine beef calves, 3 to 6 months old, were purchased and housed in a separate pen in the aforementioned experimental barn unit. Within a week several of these calves developed temperatures varying from 104° to 106°F, slight cough, moderate nasal exudate, and considerable mucus in the stools. These animals maintained normal food intake and recovered without treatment in approximately 1 week.

A viral agent, cytopathic for bovine kidney cells grown *in vitro*, was recovered from (i) fecal samples collected from four of the latter calves during the late stages of the illness and also 3 months after recovery; (ii) a pool of fecal samples collected 15 February 1957 from the two cows, one of which aborted; (iii) samples of ascites fluid, stomach fluid, and placental fluid collected from the aborted fetus; and (iv) a pool of fecal samples from calves in another experimental unit which had fever and slight nasal exudate.

Ten pooled fecal samples collected from calves and cows with no history of illness were found free from this virus as judged by the absence of cytopathic changes in inoculated tissue cultures.

Tissue cultures of bovine kidney (*2*) were prepared in tubes of the trypsin digest method described by Youngner (*3*). The cells were grown in a nutrient medium consisting of Hank's solution with 0.5 percent lactalbumin hydrolyzate and 10 percent bovine serum. When cell sheets were formed on the glass surface, this nutrient medium was withdrawn and replaced with a maintenance medium consisting of Hank's solution containing 0.5 percent lactalbumin hydrolyzate but no serum. To both media were added 100 units of penicillin, 200 μg of streptomycin, and 100 units of mycostatin (*4*) per milliliter.

Fecal samples were suspended in phosphate-buffered saline containing 500 units of penicillin and 500 μg of streptomycin per milliliter. These suspensions were strained through a four-layer pad of cheesecloth into stainless-steel tubes which were centrifuged at 10,000 rev/min for 30 minutes in a refrigerated centrifuge. Each supernatant was subsequently diluted 1/2 with maintenance medium and dispensed in quantities of 1 ml to each of five tissue-culture tubes. After 2 to 3 hours, this inoculum was withdrawn and replaced with fresh maintenance medium. The fetal fluids which were clear and free of bacteria were diluted 1/5 with maintenance medium and dispensed in quantities of 1 ml to each of five tissue-culture tubes.

Cytopathic changes characterized by focal rounding and shrinking of cells were observed in the bovine kidney cell cultures within 12 to 24 hours. These changes progressed until all cells were affected and fell off the glass surface after 24 to 96 hours (Fig. 1). The fluid of culture tubes in which the majority of cells were affected also was considerably more acid than the fluid of the unaffected control tubes.

Ten serial passages of infected cell culture fluids carried out have regularly resulted in cytopathic cell changes as well as in increased acidity of the culture fluids. The viral agents recovered from the different sources have behaved identically in these aspects.

Replicate titrations of various tissue culture passage levels showed a $TCID_{50}$ of 10^7 to 10^{12} per milliliter. Cell cultures inoculated with the lowest dilution of infected fluids showed typical cytopathic changes as early as 4 hours after inoculation, while those inoculated with the highest dilution revealed such changes 36 to 42 hours after inoculation.

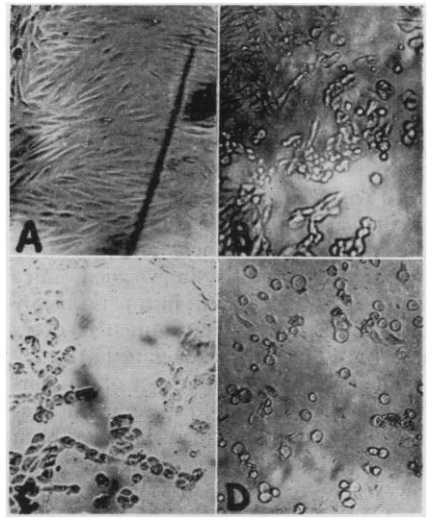

Fig. 1. Bovine kidney cells (about × 15). (*A*) Normal cells growing on the glass wall of a test tube. (*B*) Early cytopathic effect (1+) of virus growth; a number of cells have become shrunken or rounded, but most of the cell sheet appears normal. (*C*) Progression of virus-induced cytopathic changes (2 to 3+); a large number of cells are rounded or shrunken. (*D*) Final stage of cytopathic changes (4+); no normal cells remain, and most cells have fallen off the glass surface.

The increase in acidity at the time of severe or complete cell destruction varied from 0.2 to 0.4 *p*H units as compared with normal cell cultures. The viral agent replicates and produces cytopathic changes in bovine kidney cell cultures at *p*H 6.4 to 8.4. The cytopathic changes develop more slowly and seem less extensive at *p*H 6.4 to 6.6 than at *p*H 7.4 to 8.4. Considerable rounding and degeneration were observed in noninfected control cultures at *p*H 8.4.

The virus passes through a fine sintered glass filter without appreciable loss of titer. It will also pass an ultrafilter membrane (*5*) with average pore size of 0.05 to 0.08 μ, but not through a membrane with average pore size of 0.01 to 0.05 μ.

The virus, which is ether-resistant, survives storage in tissue-culture maintenance fluid (*p*H 7.6 to 7.8) at −10° to +22°C for at least 2 months, at 37°C for at least 120 hours, and at 56°C for at least 30 minutes.

Pooled preinfection and convalescent serums from the four calves, from whose feces virus was isolated, were tested for specific neutralizing antibodies against the virus. Tissue-culture neutralization tests were carried out in accordance with the method described by Paul and Melnick for paired poliomyelitis serums (*6*).

The preinfection serum pool failed to prevent cytopathic changes, while a 1/10 dilution of the convalescent serum pool prevented such changes (50 percent serum titer log = 1.4).

The virus causes neither signs of illness nor pathological tissue changes in normal suckling or weaned Swiss white mice when it is inoculated by the intraperitoneal or intranasal routes. However, the virus causes illness and extensive myocardial necrosis in weaned mice when it is inoculated intraperitoneally after 2 to 3 daily intramuscular doses (2.5 mg) of cortisone acetate (7).

The etiological significance of this particular virus in the disease manifestations in cattle described has not been established. The disease-producing capacities of the virus in various animal species will be evaluated experimentally. The possible serologic relationships between this viral agent and other cytopathogenic viral agents isolated from the alimentary tract will also be investigated—for example, infectious bovine rhinotracheitis virus (8), poliomyelitis virus (6), Coxsackie virus (9), ECHO viruses (10), ECBO viruses (11), and adenoviruses (12) (13).

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Decomposition of Actinomycin by a Soil Organism

Several studies indicate the importance of microorganisms in the inactivation of antibiotics, although few comprehensive studies have been attempted. The most extensive reports have dealt with the capability of many bacterial species to inactivate penicillin by the hydrolytic action of an enzyme, penicillinase (1). In addition, Pramer and Starkey (2) have reported the isolation from soil of a Gram-negative bacterium capable of decomposing streptomycin. Smith *et al.* (3) have observed that growing and resting cells of *Escherichia coli, Bacillus mycoides, Bacillus subtilis,* and *Proteus vulgaris* can degrade chloramphenicol by hydrolysis, reduction, oxidation, and cleavage of the molecule. Waksman and Woodruff (4), as a result of an investigation of the stability of actinomycin in fresh soil, suggested that inactivation of actinomycin under these conditions may be the result, in part, of the activities of soil microorganisms. The present report describes the isolation of an actinomycin-decomposing organism from soil and presents observations concerning the nature of, and the optimum conditions for, this reaction (5). An enzyme system, tentatively designated *actinomycinase,* appears to be involved in the degradation process.

The medium employed, consisting of mineral salts, 0.1 percent yeast extract, and actinomycin D, was a modification of one used by Pramer and Starkey (2). Microbiological assays of actinomycin in culture fluids were performed by disk diffusion (6) and streak dilution methods (7). Isolation of an actinomycin-decomposing organism was accomplished by enrichment of a barnyard soil and subsequent cultivation of samples of this soil in media containing actinomycin D. On the basis of morphological, staining, cultural, and biochemical characteristics, it was established that the responsible bacterium belonged to the genus *Achromobacter.*

Growing cells of the *Achromobacter* sp. readily decomposed approximately 560 μg of actinomycin D per milliliter of medium in shaken (24 hours) and static (64 hours) cultures under aerobic conditions. The organism degraded actinomycin D (500 μg/ml) through a *p*H range of 6.0 to 8.0, *p*H 7.5 being optimum (48 hours), and at temperatures between 20° and 37°C, 28°C being the most favorable temperature. The extent of decomposition was found to vary within the range of 85 to 4010 μg of actinomycin per milliliter of medium. Up to 1000 μg/ml was destroyed in 48 hours; decomposition of 4010 μg/ml required 84 hours.

A direct relationship was found to exist between inoculum size and the decomposition process. For example, if 7.4×10^{10} cells were employed, decom-

Table 1. Influence of age of *Achromobacter* sp. on the decomposition of actinomycin D. *Achromobacter* sp. was cultivated in actinomycin medium; at various intervals, samples of the culture medium were harvested for cell counts, microbiological assays, and resting-cell studies. For the latter purpose, washed cells of the organism were suspended in 0.06*M* phosphate buffer, *p*H 7.5; the actinomycin-D concentration was 1000 μg/ml, temperature 28°C.

Age of culture (hr)	Cell count (No./ml)	Percentage of actinomycin (500 μg/ml) remaining in culture medium	Percentage of actinomycin (1000 μg/ml) remaining after 6 hr incubation with resting cells
0	1.5×10^7	100	100
8	7.9×10^7	100	100
16	2.0×10^9	100	100
20	2.1×10^9	70	90
24	1.9×10^9	52	50
28	1.8×10^9	24	33
32	1.2×10^9	0	0

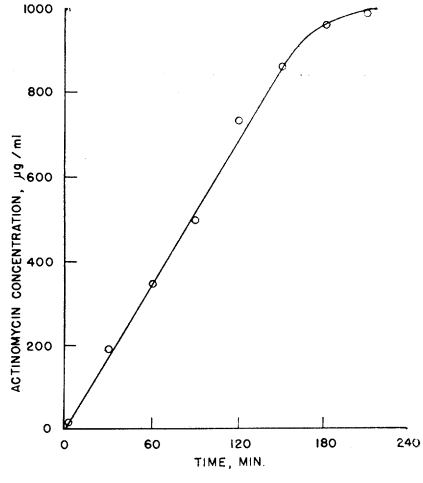

Fig. 1. The amount of Actinomysin D decomposed with time by a suspension of *Achromobacter* sp. cells. Cells were centrifuged out of actinomycin medium, washed twice with distilled water, and finally suspended in 0.06*M* phosphate buffer. One milliliter of this bacterial suspension was mixed with 1 ml of an actinomycin-D solution and 8 ml of 0.06*M* phosphate buffer, *p*H 7.5. Temperature of incubation was 28°C. Final concentration of actinomycin D was 1000 μg/ml.